

IN THE CLAIMS

Please cancel claim 45.

1. (Thrice amended) An isolated microbial cell comprising an Environmentally Limited Viability System, wherein the cell is viable in a permissive environment and non-viable in a non-permissive environment, the system comprising

(a) an essential gene, wherein expression of the gene in the cell is essential to the viability of the cell, and the essential gene is expressed when the cell is in the permissive environment and is not expressed when the cell is in the non-permissive environment, and wherein the essential gene is a copy of a [native]wild-type gene of the microbial cell; and

(b) a lethal gene, wherein expression of the gene is lethal to the cell and the lethal gene is expressed when the cell is in the non-permissive environment but not when the cell is in the permissive environment,

wherein the [native]wild-type gene is inactivated in the cell.

27. (Thrice amended) A method of making a cell strain with environmentally limited viability comprising stably introducing into a cell

(a) an essential gene, wherein expression of the gene in the cell is essential to the viability of the cell, and the essential gene is expressed when the cell is in the permissive environment and is not expressed when the cell is in the non-permissive environment, and wherein the essential gene is a copy of a [native]wild-type gene of the microbial cell;

(b) a lethal gene, wherein expression of the gene is lethal to the cell and the lethal gene is expressed when the cell is in the non-permissive environment but not when the cell is in the permissive environment,

wherein the cell strain is viable in a permissive environment and non-viable in a non-permissive environment,

wherein the [native]wild-type gene is inactivated in the cell.

30. (Four times amended) A method of inducing immunoprotection in a warm-blooded animal comprising

administering to the animal a vaccine comprising a microbial cell comprising an Environmentally Limited Viability System, wherein the cell is viable when in the animal and non-viable when outside of the animal, the system comprising

(a) an essential gene, wherein expression of the gene in the cell is essential to the viability of the cell, the essential gene is expressed when the cell is in the animal and is not expressed when the cell is